REMARKS

In the Office Action, claims 18 and 20-24 were rejected under 35 USC §112, first paragraph; claims 18 and 20-24 were rejected under 35 USC §112, second paragraph; and claim 18 is rejected under 35 USC §102. Claims 1-17 have been withdrawn; claims 18, 21 and 22 have been amended; and claims 19 and 23 have been canceled without prejudice or disclaimer. Applicants believe that the rejections have been overcome or are improper in view of the amendments and for the reasons set forth below.

At the outset, Applicants note that the Patent Office has requested resubmission of the computer-readable form of the sequence listing that Applicants' previously-submitted. Apparently, the previously-submitted computer-readable form of the sequence listing was unreadable. Therefore, Applicants are submitting concurrently herewith a computer-readable form of the sequence listing, pursuant to the Patent Office's request.

In the Office Action, claims 18 and 20-24 are rejected under 35 USC §112, first paragraph. More specifically, the Patent Office rejects claims 18, 20-22 and 24 as allegedly failing the written description requirement; and the Patent Office rejects claims 18 and 20-24 as allegedly failing the enablement requirement. Applicants believe that these rejections are improper, as discussed in detail below.

With respect to the written description rejection, Applicants believe that the subject matter as claimed was sufficiently described in the specification so as to reasonably convey to one skilled in the art that Applicants had possession of the claimed invention at the time of filing. Indeed, the specification details how the CD14 variant according to an embodiment of the claimed invention can be isolated (e.g., from milk) and, further, how the isolated CD14 variant was characterized and identified through additional experimentation. See, Specification, for example, pages 6-8 and pages 15-16. The identification experimentation included, for example, N- and C- terminal sequence analysis and an optical biosensor assay. See, Specification, pages 7 and 8. Thus, Applicants believe that the specification provides sufficient detail such that one skilled in the art would reasonably recognize that Applicants had possession of the subject matter directed to a method of prevention or treatment of a GI tract disorder by administering a CD14 variant or fragment thereof, to a patient at risk or in need of treatment as required by the pending claims at issue, namely claims 18, 20-22 and 24. Therefore, Applicants respectfully submit that the written description requirement has been satisfied.

With respect to the alleged enablement issue, the Patent Office essentially asserts that Applicants have not shown that the administration of the variant or fragment of CD14 as claimed can treat or prevent a GI tract disorder. Applicants believe that the Patent Office's position is improper.

As disclosed in the specification on pages 12-15, for example, Applicants have demonstrated that sCD14 derived from mature milk according to an embodiment of the claimed invention is biologically active and capable of mediating cell activation by lipopolysaccharides. In addition, the contribution of mmsCD14 on the stimulation of intestinal epithelial cells by Gram-negative non-pathogenic bacteria was tested. As shown in Figure 3, *E.coli* alone was unable to induce significant levels of IL-8. However, incubation of *E.coli* in the presence of either human serum or human milk was capable of inducing substantial amounts. See, specification, pages 14 and 15. Indeed, the CD14 variant as claimed can be isolated from milk, such as mature human milk, as detail in the specification, for example, on pages 15 and 16. Moreover, the specification further provides a number of examples of infant formula that include specific amounts of the CD14 variant and other constituents according to an embodiment of the claimed invention. For at least these reasons, Applicants believe that one skilled in the art should be able to readily practice the claimed invention based on what is disclosed in the Specification and further based on their knowledge and skill in the art.

In further support of this position, Applicants respectfully refer the Patent Office to the documents submitted in Exhibits A and B attached hereto. The document in Exhibit A is a report that relates to the sCD14-mediated responsiveness of HT29-cells challenged with LPS or *E.coli*. In general, the report provides data that demonstrates the release of cytokines associated with the sCD14-mediated responsiveness of HT29-cells. The data includes, for example, sICAM-1 release, TNF-α (tumor necrosis factor) *in vivo* release, MCP-1 release, and Elafin release. The attached document of Exhibit A was drafted by Ms. Anne Donnet and Ms. Karine Vidal, who are both inventors of the claimed invention. The document in Exhibit B includes a paper published by the inventors of the claimed invention and others that provides additional support to Applicants' position as discussed in further detail below.

Generally, sCD14 as disclosed in the present application in an embodiment is a regulatory molecule that, if consumed orally, modulates immune responses in the gut by controlling release of cytokines by intestinal cells. As such, Applicants believe that the soluble

CD14 variant as claimed can be effectively utilized to treat or prevent GI tract disorders, due to its general inflammatory modulating properties. More specifically, sCD14 modulates inflammatory responses that are brought about by bacteria. These may be pathogenic bacteria or bacteria naturally present in the intestines that form part of the human gut flora. As set forth in Exhibit A, GI tract disorders, in turn, can be ascribed to bacterially induced inflammations. For example, the introductory part of Exhibit A provides that the normal intestinal flora and the mucosal immune system exist in close spatial proximity and an abnormal response to the flora leads to chronic intestinal inflammation.

As previously discussed, Exhibit A provides data that demonstrates the release of cytokines associated with the sCD14-mediated responsiveness of HT29-cells. In particular, Figure 1 illustrates *in vitro* sICAM-1 release of the intestinal epithelial cell (HT29-cells). As shown, human milk together with *E. coli* or LPS (a polysaccharide typical for bacteria) provoke a prominent release of sICAM-1. However, if an anti-CD14 antibody is added (MY4), which binds to sCD14 and thus, prevents its interaction with any signaling pathway, the cytokine release is reduced. This demonstrates that sCD14 is critical in assuring that the sICAM-1 is actually released. Thus, since sICAM-1 has been suggested to contribute to the pathogenesis, for example, of coeliac disease, the effectiveness of sCD14 with respect to the development of disease, such as GI-tract disease as claimed is established.

Further, Figures 2-4 illustrate levels of TNF-α mRNA in the jejunum, ileum and in the liver, respectively, in suckling rats. Suckling rats were given different diets (i.e., mother fed, rat milk substitute (RMS), and RMS+ sCD14(recombinant)). The TNF-α mRNA-level in rats given RMS+ sCD14 is very close to that of rats given mother's milk. It is noted that the effect on TNF-α mRNA are increasingly pronounced in the liver, ileum, and jejunmum, respectively as further illustrated in Figures 2-4. Further, TNF-α, in general, has been reported to be involved in the pathogenesis of chronic hepatitis, inflammatory bowel disease, coeliac disease, necrotizing enterocolitis, allergic reactions, and the like. Thus, this further suggests that sCD14 plays a crucial role in modulating GI tract diseases as claimed by its influence on cytokines, such as TNF-α synthesis.

In addition, Exhibit A demonstrates the role of sCD14 with other important cytokines, such as Elafin and MCP-1 as illustrated in Figures 5 and 6. In general, it has been reported that these types of cytokines have an effect on a variety of GI tract disorders. Moreover, the

publication in Exhibit B reports findings that indicate "a sentinel role for sCD14 in human milk during bacterial colonization of the gut, and suggest that m-sCD14 may be involved in modulating local innate and adaptive immune responses, thus controlling homeostasis in the neonatal intestine." See, Exhibit B, Abstract.

In view of same, Applicants believe that Exhibits A and B demonstrate that soluble sCD14 in human milk can modulate *in vitro* and *in vivo* the release of cytokines provoked by pathogenic or intestinal bacteria. This suggests that soluble sCD14 can be effectively utilized to prevent and/or treat GI tract disorders, particularly those directly and indirectly linked to inflammatory conditions. Therefore, Applicants believe that this provides further support with respect to Applicants' position that one skilled in the art should be able to readily practice the claimed invention.

Based on at least these reasons, Applicants believe that the written description and enablement requirements pursuant to 35 U.S.C.§112, first paragraph have been satisfied. Accordingly, Applicants respectfully submit that the rejections of claims 18 and 20-24 under 35 U.S.C.§112, first paragraph, be withdrawn. Applicants note for the record that claim 23 has been cancelled and thus the rejection under 35 USC §112, first paragraph, with respect to same, has been rendered moot.

In the Office Action, claims 18 and 20-24 have been rejected under 35 USC §112, second paragraph. In response, claims 21 and 22 have been amended as previously discussed. Applicants believe that the amendments were made for clarification purposes and thus, were not intended to disclaim or cancel any claimed subject matter in view of same. Applicants further note that the rejection with respect to claim 23 has been rendered moot as this claim has been cancelled, as discussed above.

With respect to the rejection of claim 18 for allegedly omitting essential steps, Applicants believe that claim 18 is sufficiently defined so as to meet this requirement. However, in the spirit of cooperation, Applicants have amended claim 18 to address this issue. Again, Applicants note that this amendment was made for clarification purposes and not intended to disclaim or cancel any claimed subject matter in view of same.

The Patent Office also alleges that claims 18, 20 and 24 are indefinite for reciting ". . . which retains the bioactivity of CD14." Applicants believe that the Patent Office's position with

respect to same is improper. Indeed, support for the claim terms at issue can be found in the specification; for example, on page 2, at lines 15-28.

The Patent Office also rejects the claim term "GI tract disorders" of claim 18 as allegedly vague and indefinite. Again, Applicants believe that the Patent Office's position with respect to same is improper. Indeed, support for the claim term at issue can be found in the specification; for example, on page 9, at lines 14-23.

Based on at least these reasons, Applicants believe that the claimed invention, as defined by claims 18, 20-22 and 24 is sufficiently clear in meaning and scope. Applicants note for the record that claim 23 has been cancelled and thus, this rejection should be rendered moot with respect to same as discussed above.

Accordingly, Applicants respectfully request that the rejection of claims 18 and 20-24 under 35 USC §112, second paragraph, be withdrawn.

In the Office Action, claim 18 has been rejected under 35 USC §102 as allegedly anticipated by *Haziot et al.*. Applicants believe that this rejection is improper.

Claim 18 recites a method of treatment or prevention of a GI tract disorder which comprises the steps of administering to a patient an effective amount of a CD14 variant or fragment thereof which retains the bioactivity of CD14. The claimed invention is based upon the finding that mature milk includes an unknown variant of CD14. This variant has a high homology at the amino acid sequence with respect to serum sCD14. However, it has a different gel mobility than known serum soluble forms, where the claimed CD14 variant is the secretory product of the mammary gland epithelial cell, has a different glycosylation pattern with respect to known soluble forms of CD14, and has a unique capacity for mediating bacterial interaction with intestinal surfaces. Thus, the variant of CD14 as claimed can be effectively utilized to prevent and/or treat GI tract disorders. See, specification, page 6, lines 17-22.

In contrast, Applicants believe the cited art is distinguishable from the claimed invention. For example, the primary focus of the cited art relates to a recombinant soluble CD14 that, if injected into mice, can purportedly reduce mortality due to LPS injection (i.e., a model for sepsis).

Contrary to the Patent Office's position, the capacity of sCD14 to bind to LPS and purportedly prevent the ultimate effects of sepsis as disclosed in *Hazoit et al.* does not suggest that sCD14 can be utilized to effectively prevent or treat GI tract disorders as required by the

claimed invention. In this regard, Applicants believe that sCD14 and LPS effectively acts locally in the intestinal environment before crossing the intestinal barrier into systemic circulation. Indeed, *Hazoit et al.* merely and purportedly provides evidence that CD14 in a soluble form may have some benefit once injected into systemic circulation. This contrast the CD14 variant as claimed that Applicants believe through oral administration can effectively act locally in the gastrointestinal tract to modulate responses to LPS, prevent disease and thus may prevent passage of LPS into the blood circulation.

Based on at least these differences, Applicants believe that the cited art and the claimed invention as defined in claim 18 are distinguishable. Therefore, Applicants respectfully submit that the cited art fails to anticipate the claimed invention.

Accordingly, Applicants respectfully request that this rejection be withdrawn.

Applicants note for the record that they have not received a copy of the PTO 1449 form previously submitted by Applicants on July 30, 2003 that has the Examiner's initials indications that the references cited therein are made of record. Accordingly, Applicants kindly request a copy of same be forward to the undersigned attorney of record. If the Patent Office should have questions regarding same, Applicants respectfully request that the undersigned attorney be contacted directly.

For foregoing reasons, Applicants respectfully submit that the present application is condition for allowance and earnestly solicit reconsideration of same.

Respectfully submitted,

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Dated: December 1, 2003

Exhibit A

THE USE OF CD14 IN THE MANUFACTURE OF A NUTRITIONAL PRODUCT OR MEDICAMENT FOR THE TREATMENT OR PREVENTION OF A GI TRACT DISORDER.

The gastrointestinal tract and the liver are key organs in nutrient absorption and metabolism and often conditions affecting one of these organs have consequences for the other. For example, endotoxins derived from Gram-negative bacteria in the intestine are important in the pathogenesis of liver and systemic diseases (1). Furthermore, chronic liver diseases can influence gastrointestinal motility, which may contribute to bacterial overgrowth and in patients with ascites, to an increased risk of bacterial peritonitis (1). Several liver and biliary abnormalities are observed in patients with inflammatory bowel disease (autoimmune hepatitis). Furthermore, elevated liver transaminases are observed in many patients with celiac disease. Finally, many viral, bacterial, fungal, and parasitic diseases affect the intestine as well as the liver and the biliary tract (1).

In diseases such as inflammatory bowel diseases, Crohn's disease, ulcerative colitis, coeliac disease, intestinal bacterial overgrowth, chronic hepatitis, necrotising enterocolitis, neonatal sepsis, infectious diarrhoea, dysbalance of the intestinal microflora, allergic reactions to food and bacterial translocation from the gut, there are links between the exposure to microorganisms and the development of inflammation and other untoward immune reactions in, primarily the gastrointestinal tract, but also in the liver. Indeed, the normal intestinal flora and the mucosal immune system exist in close spatial proximity and an abnormal response to the flora leads to chronic intestinal inflammation (2). It follows therefore that any factor capable of modulating responses to bacteria or their components may be used in the prevention and/or treatment of the diseases mentioned above.

Intracellular adhesion molecule (sICAM)-1

Adhesion molecules such as ICAM-1 are important promotErs of inflammation which mediate the extravasation of leukocytes and their accumulation in inflamed tissues. Elevated levels of these molecules have been suggested to contribute to the pathogenesis of coeliac disease (3), sepsis (4,5), and allergic reactions (6, 7). It is possible that the elevated soluble ICAM-1 levels observed in inflammatory bowel diseases, Crohn's disease and ulcerative colitis (8) as well as in neonatal infection (9) and chronic hepatitis (10) reflect a host defence mechanism to limit the inflammatory response. Certainly, it has been proposed that sICAM-1 may compete with membrane-bound forms for their cognate ligands and thereby limit the rolling and stable adhesion of leukocytes (8). As such, it may limit the cellular infiltration and inflammation observed in the diseases mentioned above. The results depicted in figure 1, show that milk sCD14 in the presence of bacterial LPS induces the expression of sICAM-1 by intestinal epithelial cells (IEC) 'in vitro'. Antibody against sCD14 but not its isotype control is able to inhibit this induction.

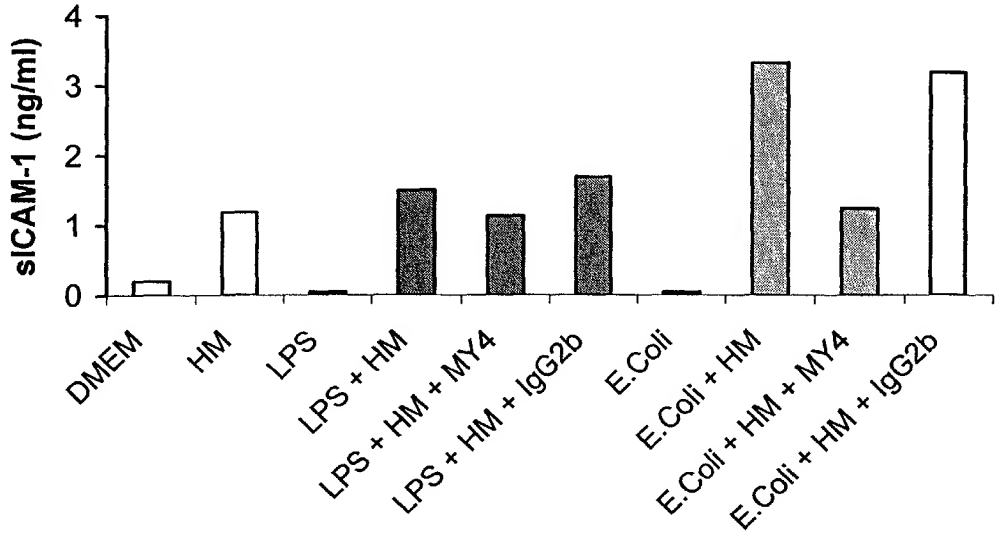

Figure 1. sCD14-mediated induction of sICAM-1 in IECs challenged by LPS or *E.Coli* bacteria. HT29 cells were cultured for 24 h in the presence of 1.7% human milk (HM) with or without 100 ng/ml of LPS or 2.5 x 10^6/ml *E. Coli*. Release of sICAM-1 was assessed by specific ELISA. The anti-CD14 mAb (MY4 at 20 μg/ml) but not its isotype control (IgG2b at 20 μg/ml) blocked the stimulation.

Tumour necrosis factor (TNF)-α

TNF-α is a major pro-inflammatory cytokine implicated in the pathogenesis of chronic hepatitis (11), inflammatory bowel disease (12), coeliac disease (13), necrotising enterocolitis (14), allergic reactions (15), sepsis (16), infectious diarrhoea (17) and bacterial translocation (18). Artificially reared suckling rats (19) underwent gastric cannulation and were machine fed a rat milk substitute (RMS) (20) or RMS containing recombinant milk sCD14 for a period of 4 days from the age of 8 days. Real-time PCR measurement of TNF-α levels in the jejunum (JEJ) (figure 2), the ileum (ILE) (figure 3) and in the liver (figure 4) revealed that artificially rats had higher levels of the cytokine in these tissues when compared to naturally suckling rat pups. However, supplementation of the RMS with CD14 reduced the TNF-α levels at all sites to a level which was closer to that of the mother-fed animals.

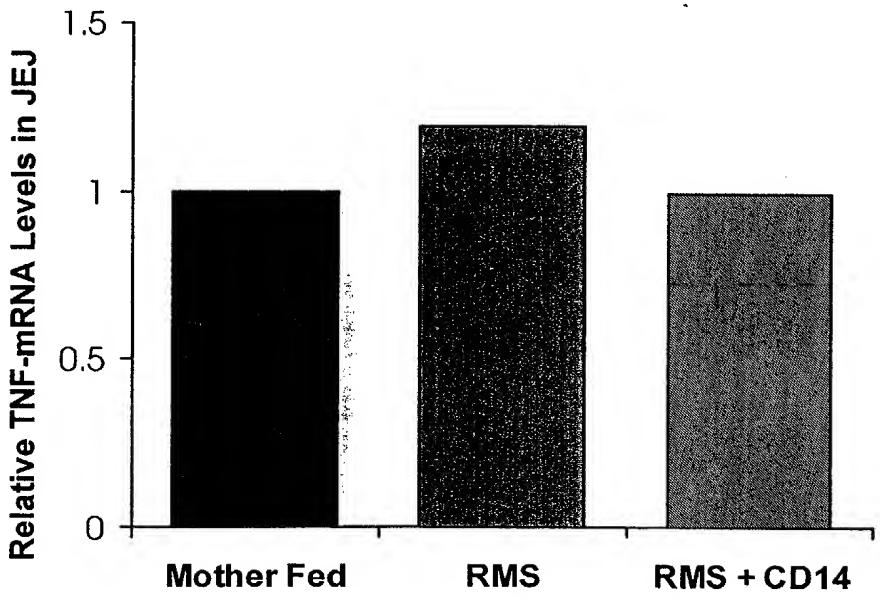

Figure 2. TNF-α mRNA levels in the jejunum (JEJ) of neonatal rats fed with mother's milk (Mother Fed), rat milk substitute (RMS) or RMS containing recombinants milk sCD14.

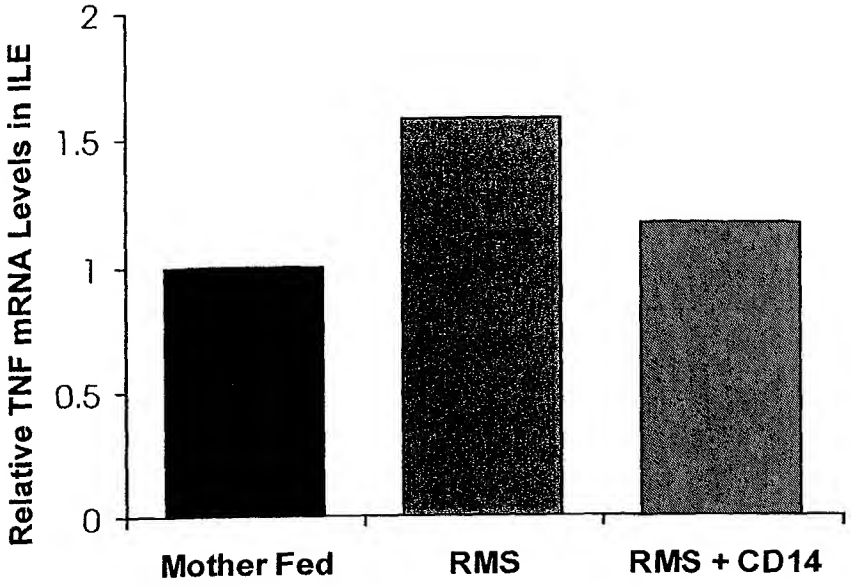

Figure 3. TNF-α mRNA levels in the ileum (ILE) of neonatal rats fed with mother's milk (Mother Fed), rat milk substitute (RMS) or RMS containing recombinants milk sCD14.

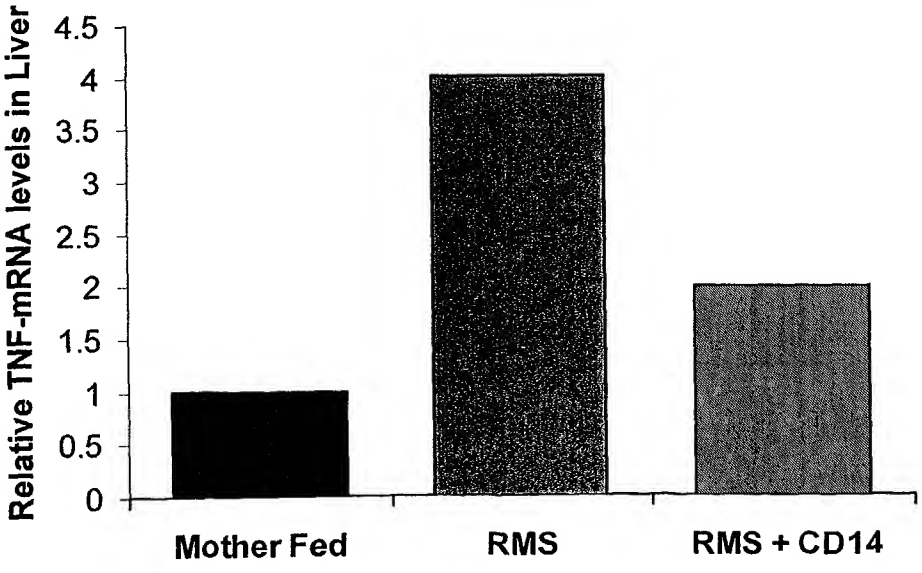

Figure 4. TNF-α mRNA levels in the liver of neonatal rats fed with mother's milk (Mother Fed), rat milk substitute (RMS) or RMS containing recombinants milk sCD14.

Elastase-specific inhibitor (ELAFIN)

ELAFIN is an anti-serine protease and anti-microbial molecule present at mucosal surfaces. It is upregulated by 'alarm signals' such as bacterial lipopolysaccharides, and pro-inflammatory cytokines and has been shown to be active against Gram-positive and Gram-negative bacteria (21). In addition, it has been suggested to play a role in the regulation of inflammation, host defence against infection, tissue repair and extracellular matrix synthesis (22). More specifically, it may have a dual function of promoting up-regulation of local innate immunity while simultaneously down-regulating potentially unwanted systemic inflammatory responses (23).

The results depicted in Figure 5, show that milk sCD14 in the presence of bacterial LPS induces the expression of ELAFIN by intestinal epithelial cells (IEC) 'in vitro'. An antibody against sCD14, but not its isotype control, is able to inhibit this induction.

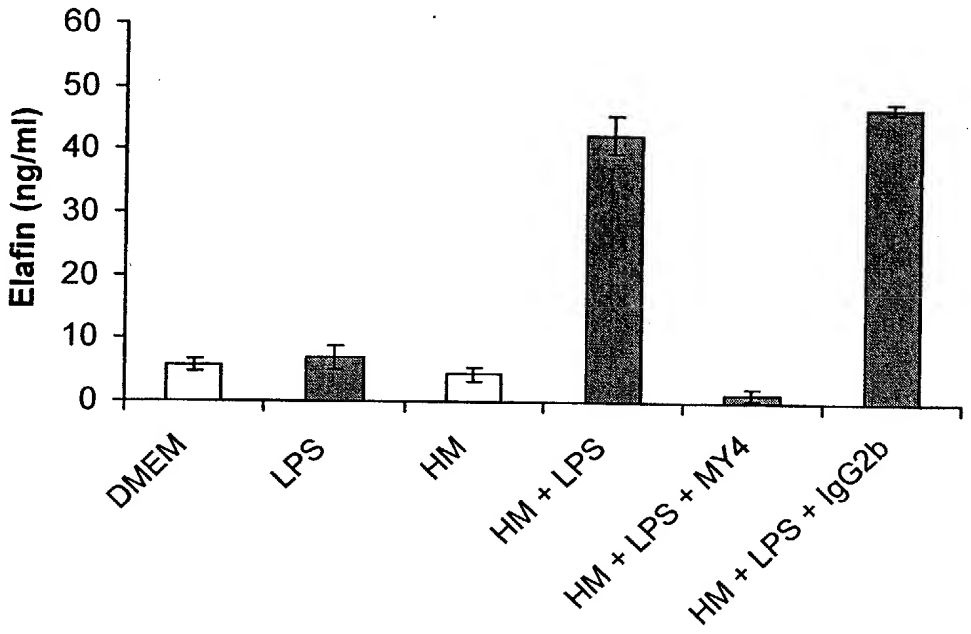

Figure 5. sCD14-mediated induction of Elafin in IECs challenged by LPS. HT29 cells were cultured for 24 h in the presence of 1.7% human milk (HM) with or without 100 ng/ml of LPS. Release of Elafin was assessed by specific ELISA. The anti-CD14 mAb (MY4 at 20 μg/ml) but not its isotype control (IgG2b at 20 μg/ml) blocked the stimulation.

Monocyte chemoattractant protein-1 (MCP-1)

The C-C chemokine MCP-1 is a potent monocyte activator and has been associated with monocytic infiltration in several inflammatory diseases (24). Indeed, it has been demonstrated that MCP-1 through binding to the CCR2 receptor, is as a major mediator of macrophage recruitment and host defense against bacterial pathogens (24).

The results depicted in Figure 6, show that milk sCD14 in the presence of the bacteria *E. coli* induces the expression of MCP-1 by intestinal epithelial cells (IEC) 'in vitro'. An antibody against sCD14, but not its isotype control, is able to inhibit this induction.

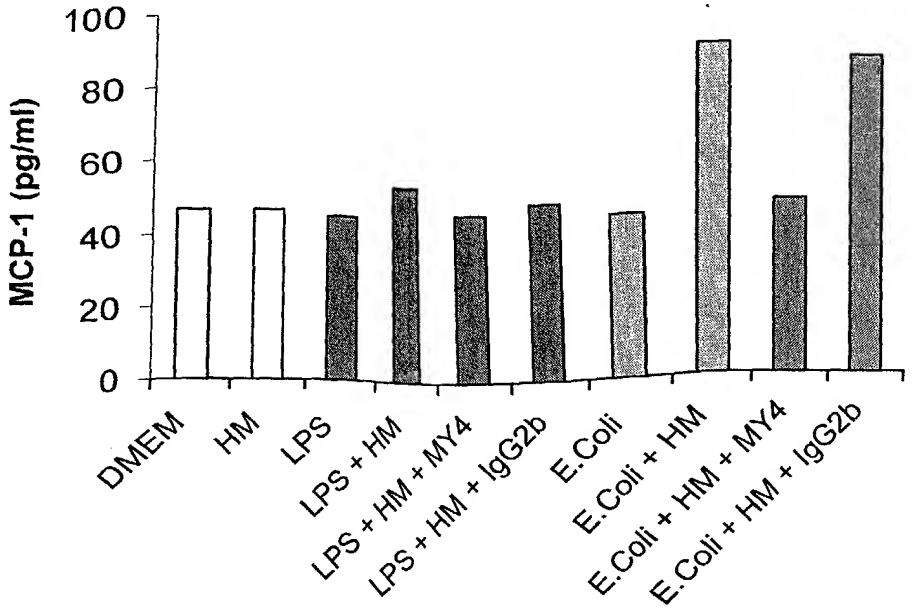

Figure 6 . sCD14-mediated induction of MCP-1 in IECs challenged by LPS or the bacteria *E.Coli*. HT29 cells were cultured for 24 h in the presence of 1.7% human milk (HM) with or without 100 ng/ml of LPS or 2.5 x 10^6/ml *E. Coli*. Release of MCP-1 was assessed by specific ELISA. The anti-CD14 mAb (MY4 at 20 µg/ml) but not its isotype control (IgG2b at 20 µg/ml) blocked the stimulation.

REFERENCES

1. Zeuzem, S. Gut-liver axis. Int-J-Colorectal-Dis. (2000) 15 : 59-82.
2. Heller, F, Duchmann,R. Intestinal flora and mucosal immune responses. Int. J Med. Microbiol. (2003) 293: 77-86.
3. Sturgess, RP, Macartney, JC, Makgoba, MW, Hung, CH, Haskard, DO, Ciclitira, PJ. Differential upregulation of intercellular adhesion molecule-1 in coeliac disease. Clin. Exp. Immunol. (1990) 82: 489-92.
4. Cuschieri, J, Gourlay, D, Garcia, I, Jelacic, S, Maier, RV. Modulation of endotoxin-induced endothelial function by calcium/calmodulin-dependent protein kinase. Shock, (2003) 20: 176-82.
5. Cuschleri, J, Gourlay, D, Garcia, I, Jelacic, S, Maier, RV. Endotoxin-induced endothelial cell proinflammatory phenotypic differentiation requires stress fiber polymerization. Shock, (2003) 19: 433-9.
6. Ciprandi, G, Tosca, MA, Cosentino, C, Riccio, AM, Passalacqua, G, Canonica, GW. Effects of fexofenadine and other antihistamines on components of the allergic response: Adhesion molecules. J Allergy Clin. Immunol. (2003) 112(4 Suppl): S78-82.
7. Osterlund, P, Smedberg, T, Schroder, J, Jarvinen, KM. Expression of intercellular adhesion molecules on circulating lymphocytes in relation to different manifestations of cow's milk allergy. Clin Exp Allergy (2003) 33: 1368-73.
8. Goke, M, Hoffmann, JC, Evers, J, Kruger, H, Manns, MP.Elevated serum concentrations of soluble selectin and immunoglobulin type adhesion molecules in patients with inflammatory bowel disease. J Gastroenterol. (1997) 32: 480-6.
9. Apostolou, M, Dimitriou, H, Kaleyias, J, Perdikogianni, C, Stiakaki, E, Costalos, C, Kalmanti, MC. Levels of soluble ICAM-1 in premature and full-term neonates with infection. Mediators Inflamm. (2002) 11: 95-8.
10. Capra, F, De Maria, E, Lunardi, C, Marchiori, L, Mezzelani, P, Beri, R, Gabrielli, G B. Serum level of soluble intercellular adhesion molecule 1 in patients with chronic liver disease related to hepatitis C virus: A prognostic marker for responses to interferon treatment. J Infect. Dis. (2000) 181: 425-31
11. Tai, DI, Tsai, SL, Chen, TC, Lo, SK, Chang, YH, Liaw, YF. Modulation of tumor necrosis factor receptors 1 and 2 in chronic hepatitis B and C: the differences and implications in pathogenesis. J Biomed. Sci. (2001) 8: 321-7.
12. D'Haens, G. Anti-TNF therapy for Crohn's disease. Curr. Pharm. Des. (2003) 9: 289-94.
13. Przemioslo, RT, Kontakou, M, Nobili, V, Ciclitira, PJ. Raised pro-inflammatory cytokines interleukin 6 and tumour necrosis factor alpha in coeliac disease mucosa detected by immunohistochemistry. Gut (1994) 35: 1398-403.
14. Tan, X, Hsueh, W, Gonzalez-Crussi, F. Cellular localization of tumor necrosis factor (TNF)-alpha transcripts in normal bowel and in necrotizing enterocolitis. TNF gene expression by Paneth cells, intestinal eosinophils, and macrophages. Am. J-Pathol. (1993) 142: 1858-65.

Exhibit B

Brief Definitive Report

Innate Recognition of Bacteria in Human Milk Is Mediated by a Milk-derived Highly Expressed Pattern Recognition Receptor, Soluble CD14

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Abstract

Little is known about innate immunity to bacteria after birth in the hitherto sterile fetal intestine. Breast-feeding has long been associated with a lower incidence of gastrointestinal infections and inflammatory and allergic diseases. We found in human breast milk a 48-kD polypeptide, which we confirmed by mass spectrometry and sequencing to be a soluble form of the bacterial pattern recognition receptor CD14 (sCD14). Milk sCD14 (m-sCD14) concentrations were up to 20-fold higher than serum sCD14 from nonpregnant, pregnant, or lactating women. In contrast, lipopolysaccharide (LPS)-binding protein was at very low levels. Mammary epithelial cells produced 48-kD sCD14. m-sCD14 mediated activation by LPS and whole bacteria of CD14 negative cells, including intestinal epithelial cells, resulting in release of innate immune response molecules. m-sCD14 was undetectable in the infant formulas and commercial (cows') milk tested, although it was present in bovine colostrum. These findings indicate a sentinel role for sCD14 in human milk during bacterial colonization of the gut, and suggest that m-sCD14 may be involved in modulating local innate and adaptive immune responses, thus controlling homeostasis in the neonatal intestine.

Key words: innate immunity • neonatal immunity • mucosal immunity • intestinal immune response • breast-feeding

Introduction

After birth, the sterile fetal intestine is heavily colonized mainly by *Escherichia coli* and *Streptococci* (1). The predominance of endotoxin (LPS)-producing Gram-negative bacteria may contribute to the pathogenesis of a variety of immune and inflammatory neonatal conditions, such as necrotizing enterocolitis and gut-originated sepsis (2). However, the epithelial layer, together with intraepithelial and lamina propria immunocompetent cells, coordinates local innate and adaptive immune responses, thus maintaining gut homeostasis (3).

Importantly, it has been demonstrated that breast-fed newborns experience a lower incidence of gastrointestinal infections and inflammatory, respiratory, and allergic diseases (4–6). Protection by milk has been variously ascribed to maternal immunocompetent cells, immunoglobulins, immune reactive peptides, antiinfectious oligosaccharides, growth factors, cytokines, lysozyme, lactoferrin, and complement components (7). However, we speculated that more specific factors may be present in milk that would continuously sense and signal to the neonate the presence of microorganisms, thus contributing to maintain intestinal immune homeostasis.

The bacterial pattern recognition receptor (PRR) CD14 plays a pivotal role in the recognition of and cell activation induced by microbial cell wall components of Gram-negative and Gram-positive bacteria as well as mycobacteria (8, 9). Two soluble forms of CD14, sCD14α and sCD14β, are found in normal human plasma at a concentration of 2–3

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μg/ml (10, 11). sCD14 binds to whole bacteria and bacterial cell wall components, and mediates bacterial-induced activation of cells that do not express membrane-bound CD14 as well as CD14-bearing cells (12–14).

In view of this PRR activity of CD14, we tested whether sCD14 is present in human milk and contributes to the innate immune mechanisms controlling gut homeostasis in newborns.

Materials and Methods

Reagents. Abs: anti-human CD14 mAb MY4 (IgG2b; Beckman Coulter) and MEM-18 (IgG1κ; provided by Dr. V. Horejsi, Academy of Sciences, Czech Republic) and their isotype-matched controls MOPC 141 and MOPC 21, respectively (Sigma-Aldrich). The rabbit anti-CD14 Ab (Sanofi) used in Western blot analyses was raised in animals immunized with human recombinant sCD14 and cross-reacts with bovine and porcine CD14. LPS and nonpathogenic *E. coli* serotype O55:B5 were from Sigma-Aldrich.

Human Milk and Serum, Infant Milk Formulas, and Bovine Milk. Human serum and breast milk samples were collected from healthy donors after written consent. Milk was processed within 2 h of collection. After centrifugation, the cellular pellet was used for analysis of macrophage-derived sCD14, and the milk samples were kept at −80°C until testing for sCD14 and LPS-binding protein (LBP). Commercial infant milk formulas were tested for sCD14 content and bioactivity after reconstitution. Commercial bovine milk (bottled, pasteurized cows' milk) was kept at 4°C before testing for sCD14. Bovine colostrum was obtained from animals kept in local barns. sCD14 (Immuno-Biological Laboratories) and LBP (Hycult Biotechnology) concentrations were determined by ELISA.

Western Blot Analysis. Samples were separated by 12.5% SDS-PAGE under reducing conditions (PhastSystem®; Amersham Pharmacia Biotech, or Mini Protean II; Bio-Rad) and analyzed by Western blotting as described (11).

Isolation and Purification of m-sCD14, Mass Spectrometry, and Sequencing. m-sCD14 was isolated from a pool of 3-mo-postpartum breast milk samples by affinity chromatography in a Sepharose 4B precolumn (Amersham Pharmacia Biotech) in tandem to a CN-Br Sepharose 4B–coupled MEM-18 mAb (20 mg) affinity column. The bound protein was eluted (PBS/50 mM diethylamine, pH 11.5), dialyzed against PBS, and concentrated. Some preparations were further purified by gel filtration on a Superose 12 column (fast protein liquid chromatography [FPLC]; Amersham Pharmacia Biotech). The purity of the isolated protein was confirmed by SDS-PAGE followed by silver staining, which showed a single 48-kD polypeptide. For mass spectrometric analyses, purified m-sCD14 was reduced, alkylated, and digested with porcine trypsin and lysyl endopeptidase C (Lys-C) according to standard protocols (15). Mass spectrometry (MS) and tandem MS (MS/MS) of peptides were recorded in a nanoelectrospray Q-TOF mass spectrometer (Micromass). Liquid chromatography/MS was performed with an UltiMate NanoHPLC pump equipped with a NanoColumn PepMap reverse phase (RP)HPLC (LC Packings) on line with the nanoelectrospray source. NH_2-terminal sequencing was performed in an Applied Biosystems sequencer according to the Edman degradation protocol.

Cell Lines, Cell Activation, and Cytokine and Chemokine Determinations. The human colon carcinoma epithelial cell lines HT29 and SW620, myeloid cell line U937 (American Type Culture

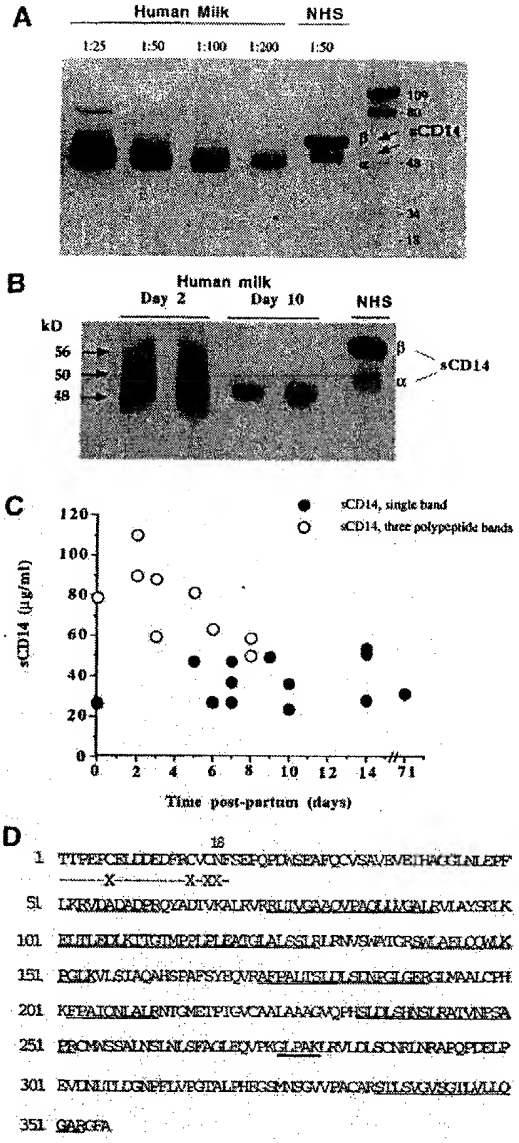

Figure 1. Detection and characterization of sCD14 in human milk. (A) Milk samples (1:25 to 1:200 dilutions) taken after the first week postpartum and normal human serum (NHS) were tested for sCD14 by Western blotting. Shown is the result of one sample representative of 10 donors. (B) Analysis of m-sCD14 in milk samples taken at day 2 or day 10 postpartum from the same mother. Samples from two donors are shown. NHS, normal human serum. (C) m-sCD14 levels determined by ELISA in multiple samples taken from 10 donors at different times postpartum. Values correspond to the mean of triplicate determinations (SD ≤ 6%). The m-sCD14 molecular pattern of each sample was determined by Western blotting and is indicated by the symbols. (D) NH_2-terminal sequence (dashed line) and mass spectrometric analysis followed by amino acid sequencing (solid line) of 48-kD m-sCD14 tryptic peptides showing homology with the predicted sequence from monocyte CD14 cDNA. Thick solid line underlines a peptide analyzed only by mass spectrometry. X, not determined.

Collection), astrocytoma line U373 MG, and breast adenocarcinoma cell line with characteristics of differentiated mammary epithelium, MCF-7 (European Collection of Animal Cell Cultures), were cultured as indicated by the purveyor. To test for CD14 expression, MCF-7 cells were cultured in AIM-V (GIBCO BRL) serum-free medium (5×10^5 cells/ml) for 72 h before analysis of cell lysates and filtered supernatants by Western blotting. For cell activation, 90% confluent cultures of HT29 (in 24-well plates), SW620, and U373 (in 96-well plates) cells and the U937 cell line (5×10^5 cells/ml) were washed and cultured for an additional 24 h in medium supplemented with normal human serum, FCS, or milk in the absence or presence of *E. coli* or *E. coli* LPS before culture supernatants were tested for TNF-α (Diaclone), epithelial neutrophil activator (ENA)-78 (R&D Systems), IL-6, or IL-8 by ELISA (IL-6– and IL-8–specific matched-pair Abs were from Immunokontact).

Results

Detection of sCD14 in Human Milk. Western blot analysis using anti-CD14–specific Abs of breast milk samples ($n = 10$) taken after the first week postpartum showed a strong single 48-kD polypeptide band (Fig. 1 A). The parallel serum samples had the typical doublet of sCD14β (56-kD) and sCD14α (50-kD) polypeptides, as described (11). The sCD14 pattern in milk from the same subject at early (<6 d), and late (>8 d) times postpartum was different (Fig. 1 B). Most of the early samples had a complex sCD14 pattern with three polypeptide bands: a strong 48-kD sCD14 polypeptide and two slower migrating polypeptides of ~50 and 56 kD, which resembled serum sCD14α and β, respectively. The later samples from the same subject had a single 48-kD sCD14 band.

Levels of m-sCD14 in multiple milk samples from 10 donors, taken at different times postpartum (Fig. 1 C), were very high (52.9 ± 24.0 μg/ml; $n = 22$) compared with those reported for normal serum (2-3 μg/ml; reference 10). The highest m-sCD14 levels were detected in the relatively early samples (≤6 d, 67.09 ± 27.61 μg/ml; $n = 10$). The majority of these early samples showed the three-sCD14 polypeptide pattern (Fig. 1 B). m-sCD14 concentration declined over the time to values of 41.12 ± 11.91 μg/ml ($n = 12$; ≥7 d postpartum). The serum sCD14 concentrations in mothers after three and eight mo of pregnancy and after three and six mo postpartum during lactation (Table I) remained similar to those reported for normal donors (pregnancy, 3.71 ± 0.57 μg/ml; $n = 20$, and postpartum, 3.76 ± 0.56 μg/ml; $n = 20$). However, the milk samples from the same mothers showed significantly higher levels of m-sCD14 compared with serum sCD14 (14.84 ± 6.40 μg/ml vs. 3.76 ± 0.56 μg/ml; $n = 20$; $P < 0.001$; milk vs. serum, three and six months postpartum). Thus, the high levels of m-sCD14 did not reflect a systemic increase in sCD14 during pregnancy and postpartum.

LBP, the protein that accelerates the binding of LPS to sCD14 (16), was at a 1000-fold lower concentration in milk than in serum of the same donor (Table I), in marked contrast with the high levels of m-sCD14. Together, these results indicated that sCD14 but not LBP is specifically expressed at high concentration in human milk.

Biochemical Characterization of m-sCD14. The NH_2-terminal sequence of the 48-kD milk polypeptide (Fig 1 D), present during most of the lactating period, was identical to that reported for sCD14 from urine of nephrotic patients and monocyte-derived CD14 (10, 17). No signal was detected at position 18, which was consistent with an *N*-glycosylation at asparagine, as predicted by the CD14 cDNA (17). Analysis of tryptic peptides from the purified material by Q-TOF mass spectrometry and sequencing indicated high homology with the amino acid sequence predicted by the CD14 cDNA (Fig 1 D). These results confirmed the identity of m-sCD14 and indicated that differences in glycosylation may account for the difference in electrophoretic mobility and pattern between 48-kD m-sCD14 and serum sCD14. Furthermore, m-sCD14 did not incorporate into lipid micelles or elute with the void volume when subjected to size exclusion chromatography (not shown), indicating that it lacks the glycolipid tail (18).

Cellular Origin of m-sCD14. The extremely high concentration of sCD14 in milk and the presence, during most of the lactating period, of a single 48-kD sCD14 polypep-

Table I. *Levels of sCD14 and LBP in Human Serum and Milk during Pregnancy and Postpartum*

		sCD14		LBP	
		Serum	Milk	Serum	Milk
		μg/ml		*μg/ml*	
Pregnancy	3 mo	3.50 ± 0.48	–	13.52 ± 4.58	–
	8 mo	3.92 ± 0.61	–	17.21 ± 3.01	–
Postpartum	5 d	–	20.10 ± 8.74	–	0.03 ± 0.01
	1 mo	–	12.06 ± 4.77	–	0.01 ± 0.02
	3 mo	3.71 ± 0.59	12.16 ± 3.75	10.65 ± 2.39	0.01 ± 0.01
	6 mo	3.82 ± 0.47	15.05 ± 4.08	8.68 ± 1.27	0.01 ± 0.01

Data represent mean ± SD of 10 (sCD14) and 8 (LBP) samples tested by ELISA.

tide contrasted with the level and pattern of monocyte-derived sCD14 present in serum (reference 11; Table I and Fig. 1). We therefore asked whether mammary gland cells produced m-sCD14. Flow cytometric analysis of the mammary epithelial cell line MCF-7 did not show surface expression of CD14 (not shown). However, cell lysates and culture supernatants from serum-free cultured cells showed CD14 polypeptide bands (Fig. 2 A). The culture supernatant showed a 48-kD sCD14 polypeptide similar to that detected in relatively late milk samples. The cell lysate showed two closely migrating CD14 polypeptides of ~48 kD, one of them of slightly faster mobility, most likely representing a precursor of the mature sCD14 glycoprotein. Supernatants of serum-free cultured milk-derived macrophages showed low levels of the typical serum sCD14α and sCD14β isoforms (not shown).

m-sCD14 Is Absent from Infant Milk Formulas and Commercial (Cows') Milk. We asked whether presence of m-sCD14 is limited to breast milk. sCD14 was not detected in the infant milk formulations (powdered milk) and commercial cows' milk (bottled, pasteurized milk) tested. However, it was detectable in bovine colostrum and plasma (Fig. 2 B).

Endotoxin and Whole Bacteria Induce Cell Activation by a Human Milk–mediated m-sCD14–dependent Mechanism. Next, we asked whether m-sCD14 could function as a mediator of endotoxin activation of cells that lack cell membrane CD14, as demonstrated for serum sCD14 (13). Human milk mediated LPS-induced IL-6 production by the CD14-negative cell line U373 in a dose-dependent manner (Fig. 3 A). Two anti-CD14 mAbs (MY4, MEM-18) abrogated the IL-6 production, suggesting that m-sCD14 was critically involved in this activity. Importantly, bovine colostrum and plasma, but not the powdered milk tested, mediated IL-6 production by LPS-stimulated U373 cells (not shown). Human milk also mediated LPS-induced IL-8 production by the CD14-negative myeloid cell line U937, and this effect was blocked by MY4 (Fig. 3 B). We confirmed that m-sCD14 was responsible for the milk-mediated cell activation by LPS, as purified m-sCD14 was able to reproduce the effect of milk (Fig. 3 C).

We also tested the capacity of intestinal epithelial cell (IEC) lines (HT-29 and SW620 lines) to produce immune and proinflammatory molecules when challenged by endotoxin or whole bacteria in the presence of human milk.

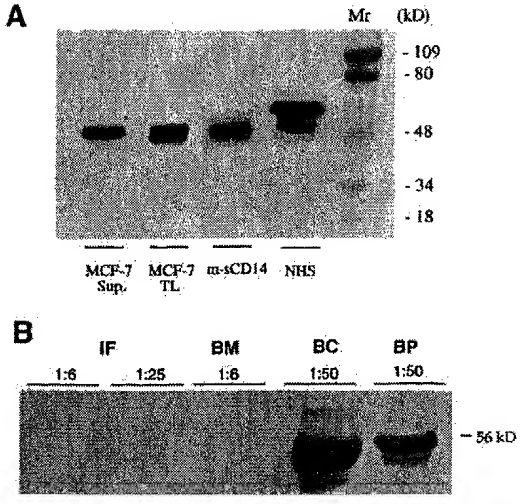

Figure 2. 48-kD sCD14 production by mammary epithelial cells and absence from commercial infant milk formulas and cows' milk. (A) Detection of sCD14 polypeptides by Western blotting in culture supernatants (Sup.) and total lysates (TL) from serum-free medium–cultured mammary epithelial cell line MCF-7. Parallel analysis of m-sCD14 in milk and normal human serum (NHS) is shown. Mr, relative molecular mass. (B) Infant milk formulas (IF, 1:6 and 1:25 dilutions), cows' milk (bottled milk [BM]), bovine colostrum (BC), and bovine plasma (BP) were tested for sCD14 by Western blotting. Shown is a representative result of 5 IF, 8 BM, 2 BC, and 10 BP.

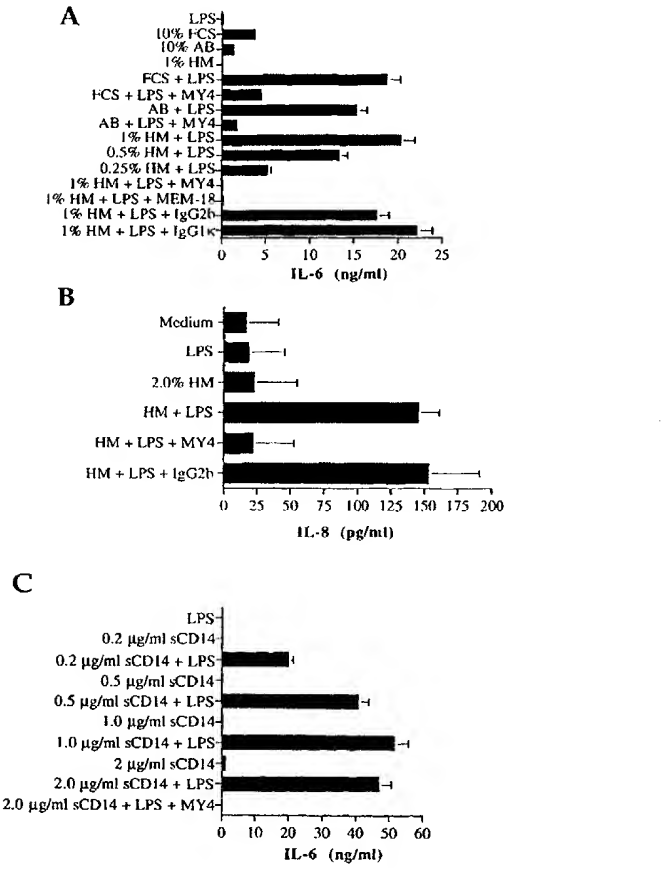

Figure 3. m-sCD14 mediates LPS activation of CD14-negative cells. (A and B) IL-6 and IL-8 production by the astrocytoma cell line U373 (A) and the myeloid cell line U937 (B), respectively, stimulated with *E. coli* LPS (100 ng/ml) in medium supplemented with FCS, human AB serum (AB), or human milk (HM). The anti-CD14 mAb MY4 and MEM-18 but not their isotype-matched controls (20 μg/ml) blocked the LPS-induced cell activation. (C) IL-6 production by LPS stimulated U373 cells in the presence of different amounts of purified milk-derived sCD14. All cytokines were tested by ELISA. Results in A and C are means ± SD of triplicate cultures of one experiment representative of four; results in B are the mean ± SD of three independent experiments.

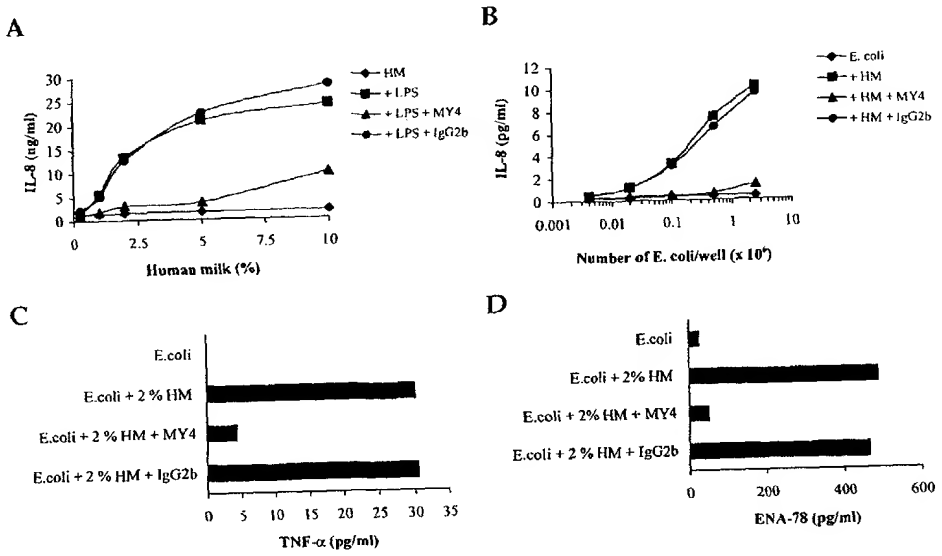

Figure 4. IECs can be activated by LPS or whole bacteria by an m-sCD14–dependent mechanism. (A–D) IL-8, TNF-α, and ENA-78 production was tested by ELISA in supernatants of HT29 and SW620 cell lines cultured in medium supplemented with human milk (HM) and stimulated with *E. coli* LPS or varying numbers of whole *E. coli* (C and D; 2.5×10^6 *E. coli*). The anti-CD14 mAb MY4 (IgG2b) but not its isotype-matched control blocked LPS and bacterial stimulation. Representative results with HT29 cells are shown.

IL-8 production by LPS-activated IEC was mediated by human milk, and this activity was inhibited by an anti-CD14 mAb (Fig. 4 A). Similarly, human milk mediated IL-8 production by IECs challenged with *E. coli*, and this effect was blocked by an anti-CD14 mAb (Fig. 4 B). IECs also responded to bacteria by producing TNF-α and the CXC chemokine, ENA-78; these responses were inhibited by anti-CD14 mAb (Fig. 4, C and D). The m-sCD14–mediated IEC activation by bacteria appears to be selective, as expression of other immune response molecules (i.e., IL-7, IL-15, IL-18, MHC class I and class II, and CD80) was not affected (data not shown).

Discussion

Here, we demonstrate that the soluble form of the receptor critically involved in innate recognition of bacteria, CD14, is present and at high concentration in human milk. Furthermore, we show that m-sCD14 can mediate IEC cell activation induced by endotoxin and whole bacteria, resulting in the production of potent immune response and proinflammatory mediators. Importantly, we found that differentiated mammary epithelial cells are able to produce sCD14 whose molecular pattern is identical to that of sCD14 present in human milk during most of the lactating period. This, together with the lack of correlation between the high concentration of m-sCD14 and the corresponding serum sCD14 levels during pregnancy and lactation, point at the mammary gland epithelium as the main source of m-sCD14. It should be noted that milk-derived macrophages showed expression of the typical serum sCD14α and β isoforms. These sCD14 polypeptides were similar to those which, in addition to the strong 48-kD sCD14 polypeptide band, are part of the complex sCD14 pattern detectable in early milk samples (Fig. 1 B). Thus, it is possible that milk macrophage–derived sCD14 contributes to the total pool of m-sCD14 within the first week postpartum. Additionally, serum sCD14 may reach the milk by the paracellular pathway, which allows components of the interstitial space to pass between the alveolar epithelial cells (7). This process does not operate during full lactation, explaining the disappearance of sCD14α and β isoforms from late milk samples.

At first sight, it was surprising to find very low levels of LBP in milk, in view of the role that this serum protein plays in facilitating the interaction of LPS with CD14. However, the low concentration of LBP may be beneficial by leading to a more controlled immune response against bacteria, as it was demonstrated that sCD14 can mediate CD14-negative cell activation by LPS in the absence of LBP, albeit with slower kinetics (16).

We consider two possible roles for m-sCD14: (i) in protection of the mammary gland against bacterial infection during lactation, and (ii) in protection of the neonatal gut. We have so far focused on the latter role.

The data show that m-sCD14–mediated in vitro stimulation of IECs by LPS and Gram-negative bacteria results in the release of molecules involved in cellular recruitment and innate defense at the site of infection. However, a number of mediators of epithelial–T cell communication were not affected. These findings indicate that the m-sCD14–mediated gut response is selective and modulated. Indeed, our findings pose the question of why, given the heavy bacterial colonization of the newborn's intestine, the presence at high concentration of m-sCD14 does not result in an excessive and deleterious immune response? One possibility is that m-sCD14 loses bioactivity during transit through the newborn's stomach and proximal intestine. The high load of m-sCD14 in the mother's milk may be necessary to compensate for such losses. We speculate that high concentrations of m-sCD14 are provided for the newborn to cope with the vast microbial inoculum and signal its presence to the neonatal intestine, which in turn will

initiate innate and adaptive immune responses. Modulation of these responses, thus avoiding excessive immune and inflammatory reactions, may be achieved by several contributing mechanisms in which m-sCD14 is involved, including the slow kinetics of cell activation by bacteria as a consequence of the very low levels of LBP, endotoxin tolerization (19) resulting from this regulated level of cell activation, the selective response of the IEC to bacterial challenge described here, and the production of the potent immunosuppressant prostaglandin E_2 by the LPS-stimulated lamina propria mononuclear cells, as has been suggested (20). In addition, m-sCD14 may participate in modulating the gut immune response by interacting directly (without LPS) with local T and B cells, which will cause modulation of T and B cell activation and function, as we recently described (21, 22).

In conclusion, the findings reported here indicate a sentinel role for m-sCD14 during the neonatal period, consistent with its PRR function in innate immunity, and suggest an immunomodulatory activity of m-sCD14. We propose that sCD14 in breast milk is responsible, at least in part, for the association of breast-feeding with a lower incidence of gastrointestinal infections and other inflammatory and allergic conditions, which depends on adequate stimulation of the gut immune system during microbial exposure early in life.

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References

1. Mackie, R.I., A. Sghir, and H.R. Gaskins. 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am. J. Clin. Nutr.* 69:1035S–1045S.
2. Peter, C.S., M. Feuerhahn, B. Bhonhorst, M. Schlaud, S. Ziesing, H. von der Hardt, and C.F. Poets. 1999. Necrotising enterocolitis: is there a relationship to specific pathogens? *Eur. J. Pediatr.* 158:67–70.
3. Powrie, F. 1995. T cells in inflammatory bowel disease: protective and pathogenic roles. *Immunity.* 3:171–174.
4. Howie, P.W., J.S. Forsyth, S.A. Ogston, A. Clark, and C.D. Florey. 1990. Protective effect of breast feeding against infection. *BMJ.* 300:11–16.
5. César, J.A., C.G. Victora, F.C. Barros, I.S. Santos, and J.A. Flores. 1999. Impact of breast feeding on admission for pneumonia during postneonatal period in Brazil: nested case-control study. *BMJ.* 318:1316–1320.
6. Oddy, W.H., P.G. Holt, P.D. Sly, W. Read, L.I. Landau, F.J. Stanley, G.E. Kendall, and P.R. Burton. 1999. Association between breast feeding and asthma in 6 year-old children: findings of a prospective birth cohort study. *BMJ.* 319: 815–819.
7. Neville, M.C. 1995. Lactogenesis in women: a cascade of events revealed by milk composition. *In* The Composition of Milks. R.D. Jensen, editor. Academic Press, San Diego, CA. 87–98.
8. Pugin, J., D. Heumann, A. Tomasz, V.V. Kravchenko, Y. Akamatsu, M. Nishijima, M.P. Glauser, P.S. Tobias, and R.J. Ulevitch. 1994. CD14 is a pattern recognition receptor. *Immunity.* 1:509–516.
9. Wright, S.D. 1995. CD14 and innate recognition of bacteria. *J. Immunol.* 155:6–8.
10. Bazil, V., V. Horejsi, M. Baudys, H. Kristofova, J.L. Strominger, W. Kostka, and I. Hilgert. 1986. Biochemical characterization of a soluble form of the 54-kDa monocyte surface antigen. *Eur. J. Immunol.* 16:1583–1589.
11. Durieux, J.J., N. Vita, O. Popescu, F. Guette, J. Calzada-Wack, R. Munker, R.E. Schmidt, J. Lupker, P. Ferrara, H.W. Ziegler-Heitbrock, and M.O. Labéta. 1994. The two soluble forms of the lipopolysaccharide receptor, CD14: characterization and release by normal human monocytes. *Eur. J. Immunol.* 24:2006–2012.
12. Jack, R.S., U. Grunwald, F. Stelter, G. Workalemahu, and C. Schütt. 1995. Both membrane bound and soluble forms of CD14 bind to Gram-negative bacteria. *Eur. J. Immunol.* 25: 1436–1441.
13. Frey, E.A., D.S. Miller, T.G. Jahr, A. Sundan, V. Bazil, T. Espevik, B.B. Finlay, and S.D. Wright. 1992. Soluble CD14 participates in the response of cells to lipopolysaccharide. *J. Exp. Med.* 176:1665–1671.
14. Labéta, M.O., J.J. Durieux, N. Fernandez, R. Herrmann, and P. Ferrara. 1993. Release from a human monocyte-like cell line of two different soluble forms of the lipopolysaccharide receptor, CD14. *Eur. J. Immunol.* 23:2144–2151.
15. Smith, B.J. 1997. Protein Sequencing Protocols. Humana Press, Totowa, NJ. 392 pp.
16. Hailman, E., H.S. Lichenstein, M. Wurfel, D.S. Miller, D.A. Johnson, M. Kelley, L.A. Busse, M.M. Zukowski, and S.D. Wright. 1994. Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to sCD14. *J. Exp. Med.* 79:269–277.
17. Ferrero, E., and S.M. Goyert. 1988. Nucleotide sequence of the gene encoding the monocyte differentiation antigen, CD14. *Nucleic Acids Res.* 16:4173.
18. Rooney, I.A., J.P. Atkinson, E.S. Krul, G. Sconfeld, K. Polakoski, J.E. Saffitz, and B.P. Morgan. 1993. Physiologic relevance of the membrane attack complex inhibitory protein CD59 in human seminal plasma: CD59 is present on extracellular organelles (prostasomes), binds cell membranes, and inhibits complement mediated lysis. *J. Exp. Med.* 177:1409–1420.
19. Ziegler-Heitbrock, H.W., M. Frankenberger, and A. Wedel. 1995. Tolerance to lipopolysaccharide in human blood monocytes. *Immunobiology.* 193:217–223.
20. Newberry, R.D., W.F. Stenson, and R.G. Lorenz. 1999. Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigens. *Nat. Med.* 8:900–912.
21. Rey Nores, J.E., A. Bensussan, N. Vita, F. Stelter, M.A. Arias, M. Jones, S. Lefort, L.K. Borysiewicz, P. Ferrara, and M.O. Labéta. 1999. Soluble CD14 acts as a negative regulator of human T cell activation and function. *Eur. J. Immunol.* 29:265–276.
22. Arias, M.A., J.E. Rey Nores, N. Vita, F. Stelter, L.K. Borysiewicz, P. Ferrara, and M.O. Labéta. 2000. Cutting edge: human B cell function is regulated by interaction with soluble CD14: opposite effects on IgG1 and IgE production. *J. Immunol.* 164:3480–3486.